Claims 13-29 have been canceled, without prejudice. Claims 30-46 have been added. Claims 1-12 and 30-46 are pending. Claims 1-12 have been withdrawn from consideration. Claims 30-46 are under active consideration.

Claims 26 and 27 have been rewritten to define the elected invention. The objection to claims 26 and 27 stated in paragraph 2 on page 3 of the Office Action of January 28, 2002 (Paper No.7), is moot.

The Section 101 rejection of claim 28 is moot in view of the above. The claims are submitted to define patentable subject matter.

The Section 112, first paragraph, rejection of claim 28 stated in paragraph 4 of Paper No. 7 is moot. The claims are submitted to be supported by an enabling disclosure.

The Section 112, second paragraph, rejections of claims 18, 24, 25 and 26, are moot in view o the above. The claims are submitted to be definite.

The Section 102 rejection of claims 13-20, 22-24, 26 and 28-29 over Pasarell et al. (Journal of Clinical Microbiology, July 1990, p. 1655-1657) is moot in view of the above. The claims are submitted to be patentable over the art and consideration of the following in this regard is requested.

The Examiner is understood to believe that Pasarell et al. teach the following:

(1) Concentrated culture filtrate antigens that were obtained from the genera *Alternaria, Bipolaris, Curvularia, Dactylaria, Drechslera, Embellisia, Exserohilum, Fusarium, Helminthosporium, Microsporum, Scolecobasidium and Scopulariopsis*;.

(2) The culture antigens were incubated and aerated on a rotating shaker;

(3) The concentrated culture filtrate antigens was used to immunize two New Zealand White female rabbits;

(4) An emulsion of 1 ml of each control antigen and 1 ml of Freund incomplete adjuvant was injected intramuscularly into the New Zealand rabbits. *Alternaria, Dactylaria, Drechslera, Embellisia, Fusarium, Micosporum, Scolecobasisum and Scolecobasidium and Scopulariopsis* did not have common antigens when tested against the antisera. Antigens of *Helminthosporium* only reacted with its own sera and there were no cross-reactions with any other antigens tested (p. 1656, 1st column);

(5) Antisera prepared from *E. rostratum* recognized antigens prepared from *E. holmii*;

(6) A similar result was observed with antisera prepare from *E. mcginnisii* and *E. longirostraturm*; and

(7) Common antigens are shared between the genera of *Bipolaris* and *Curvularia.*

Applicant offers the following comments in response:

(A) The term "generic" in Pasarell et al. was used to describe "genus specific" antigens (see last line in Abstract and throughout the text); whereas the term "generic" was used in the instant invention to mean detection of serum antibodies to a large panel of different fungal species (see page 4, ln 14-15), or, in other words, having a wide general application.

(B) Pasarell et al. noted that classification of some fungi is based on their appearance (see first paragraph, first sentence) which has led to some confusion. On page 1656, right column, middle, the authors noted that they have a very specific test and that some cross-reactions were not so. *Bipolaris* and *Curvularia* cross-reacted but this was because these belong to a single genus *Cochliobolus*. As an example, *Yersinia enterocolitica* and *Pasteurella pestis* used to be 2 separate genus until it was found that both really belong to the same genus, *Yersinia*.

(C) Pasarell et al. concentrated the filtrate antigens 20-25 fold by using an Amicon with a PM 10 filter. This would cause a loss of toxins and antigens of less than 10,000 molecular weight (e.g. aflatoxins, very important fungal toxins, have a molecular weight of only 400 and would have been lost by such method). The instant invention provides a far more superior method which uses undiluted, unconcentrated filtrate and provides greater applications. As but one example, the Pasarell et al. assay was used to assess rabbits injected with 20-fold concentrated antigen given with adjuvant oils intramuscularly on days 1,2, 3, 8 and intravenously on days 9, 10, 15, 16 and 17. In contrast, Applicant's assay was used to assess Gulf War Syndrome patient sera where 30% were found to have antibodies to *Chaetomium*.

(D) Although Pasarell et al. discussed some positive reactions, there certainly were a lot of "zero" reactions (see Table 1 of the Pasarell et al.).

(E) The method of detection between Pasarell et al. and the Applicant are very different. Pasarell et al. used agar immunodiffusion. In this, soluble antigens are put in one well and these migrate towards serum migrating in the other way from another well. One looks for the precipitin band which may or may not happen depending on concentrations. This method is impractical in a diagnostic laboratory for assessing several sera, whereas the ELISA method for detection of the instant invention is less subjective, more sensitive and more capable of assessing more serum samples.

In view of the foregoing, Applicant respectfully submits that Pasarell et al. does not teach the same functional characteristics of the claimed fungal or yeast culture supernatant and therefore the reference is incapable of anticipating the instant invention. That is, the cited art fails to teach each and every aspect of the presently claimed invention such that the claimed invention is not anticipated by the cited art.

The Section 102 rejection of claims 13-22, 24-25 and 29 over Calera et al. (Infection and Immunity, June 1994, p 2322-2333) is moot in view of the above. The claims are submitted to be patentable over the art and consideration of the following in this regard is requested.

The Applicant understands the Examiner to have pointed out that Calera et al. teach the following:

(1) Cell culture supernatants obtained from *Aspergillus*;

(2) *Aspergillus nidulans* antigens elicit antibodies in rabbits;

(3) *Aspergillus nidulans* antigens cross-reacted with antigens from *A. fumigatus, A. flavus, A. terreus, A. clavatus* and *A. niger*; and

(4) Screening a battery of 10 selected human serum samples from patients with aspergilloma or invasive aspergillosis demonstrated that two antigens from stationary-phase culture supernatants were consistently reactive.

The Examiner further commented that "it would be inherent that the *Aspergillus* antigens would be effective in detecting aflatoxins since aflatoxins are obtained from microorganisms of the genera *Aspergillus*." See, page 11 of Paper No. 7.

Applicant submits that there are some notable differences and limitations of the work done by Calera et al. that actually support the patentability of the presently claimed invention. Applicant offers the following comments for the Examiner's consideration in this regard:

(A) Calera et al. dealt only with proteins (3rd line of abstract and throughout the text). In contrast, Applicant found that with proteinase digestion, that only about 10-20% of the antigen was protein, or actually only a minor component (see Disclosure, page 16, Part (e): "Preliminary characterization of the fungal/yeast supernatant antigens").

(B) In the Calera et al. method, crude fungal supernatants were freeze-dried (note: Applicant found that the useful antigens are inactivated by freezing), dialyzed (anything less than 8,000 molecular weight, such as aflatoxins which are about 400 m.w., will be lost) and then concentrated with 7 volumes of acetone at –70 °C (note: acetone will remove fats and hydrophobic ring

structures such as aflatoxins). The Calera et al. method is very different from that of the presently claimed invention which accounts for differences in the respective results.

(C) In the Calera et al. study, there appears to be differences in the antigens expressed on or in the fungal cell and the antigens shed into the medium (i.e. Figure 3 and Figure 4, respectively). In contrast, in Applicant's disclosure, mice were immunized with whole killed cells, and their sera tested on ELISA plates coated with culture supernatants. That the latter were positive shows that the antigens Applicant used were similar, rather than different, on hyphal cells and supernatants.

(D) In Calera et al., the screening of human serum samples from patients with aspergilloma was actually inconsistent, not consistent. Figure 7 of the Calera et al. and the text on page 2330 (right column) showed that immunized rabbits had serum with antibodies that recognized several fungal proteins that human patient serum did not. There is the notation on the bottom of the left column that "The remaining visible bands reacted in a non-reproducible way or were also present in control sera." On page 2332, right column, top, Calera et al. note that a problem with the choice of proteins as detection antigens is their susceptibility to proteases, or enzymes that degrade the proteins, released during the growth of the culture. Also about 2/3 way down the same column, Calera et al. note "This faint response by the control sera has been reported several times (13) and could be due to the ubiquity of Aspergillus spp." At the

end of the paper, Calera et al. then note that they will concentrate on purifying protein antigens to enhance sensitivity and specificity of their detection system, suggesting that there are difficulties still to overcome.

Accordingly, it is submitted that the present invention is not anticipated by the findings published by Calera et al.

The Section 102 rejection of claims 13-18, 27 and 29 over Barros et al. (Rev. Inst. Med. Trop. S. Paulo 41(6): 343-350) is moot in view of the above. The claims are submitted to be patentable over the art and consideration of the following in this regard is requested.

The Applicant understands the Examiner to have pointed out that Barros et al. teach the following:

(1) Antigenic preparations from *Cladophilophora (Cladosporium) carrionii*;

(2) That partial chemical characterization of the antigenic preparations were obtained by determination of the levels of total lipids, protein and carbohydrates.

The Examiner further commented that "*Cladosporium* can give 'false positive' results when in diagnostic assays as evidenced by Malling et al....". See, page 13 of Paper No. 7. With respect, Applicant does not see the relevance of Barros et al. to the instant invention. The Examiner is requested to consider the following in this regard.

(A) Fungal cells are routinely pulverized, digested and chemically analyzed. There are chemical differences noted between different fungal species, however this should appear to be self-evident given that these are

different fungal species. On page 344 of Barros et al., fungal supernatants are neither saved, studied nor analyzed, and indeed cells were washed 3 times in saline to remove any of the supernatants.

(B) The false-positive results due to *Cladosporium* were prevented in Applicant's application simply by using the culture supernatants as is. While some of the fungal or yeast cultures used by Barros et al. may be the same as the claimed invention, Applicant fails to recognize the significance of the same. It appears that Barros et al. digested and characterized these fungi while Applicant uses these to develop a serological method. The applicants submit the reference fails to teach each and every aspect of the presently claimed invention such that the reference fails to anticipate the presently claimed invention..

(C) With respect to Malling et al. (cited by the Examiner on page 13 of Paper No. 7), it is noted that the antigen in Malling et al. is not described. It is produced as Pharmalgen(R) by Pharmacia, Uppsala, Sweden and its preparation is proprietary. However, the descriptions suggest that it is an "extract". This implies that whole cells are disrupted (either by physical or chemical means) and partially purified (i.e. centrifuged to remove debris). Accordingly, it will have nothing in common with the supernatant antigens of the present invention.

(D) Furthermore, Malling et al. describe how the various tests are inadequate. False positives and false negatives are common for the different tests. Taken together, one can make an educated guess if the patient is infected with *Cladosporium.* The last sentence of Malling et al. Abstract states "The

primary conclusion of the study, however, is that the final diagnosis of *Cladosporium* asthma could not be based on a positive BPT alone (due to "false positive"), but only on a combination of clinical symptoms during the spore season and a positive BPT.

(E) In contrast to the Malling et al. findings, Applicant has a single ELISA assay developed that is reliable not only for this fungus but for several fungi. It gave no false negatives for immunized mice, no false positives for Gulf War Syndrome patient sera.

Accordingly, it is submitted that Barros et al. is incapable of anticipating the presently claimed invention.

The Section 102 rejection of claims 13-18, 25 and 28-29 over Taylor et al. (J. Allergy Clin. Immunol. March 1988, Vol. 81, No. 3, p. 548-557) is moot in view of the above. The claims are submitted to be patentable over the art and consideration of the following in this regard is requested.

Taylor et al. only used a single culture of *Aspergillus fumigatus*. There is no indication if their assay is cross-reactive with different fungi, cross-reactive with other *Aspergillus* fungi, or specific.

Although Taylor et al. did use culture supernatants, such supernatants were different from those used in instant invention. In Taylor et al., Conidia (i.e. spores) were harvested from a mature culture of the fungus, these were germinated in a synthetic salts medium for only a very short time (15 hours) and then it is preserved with 4%

formaldehyde. It is likely that this preparation will only detect fungi if they are producing conidia, and not if these have growing hyphae infecting tissues.

In contrast, Applicant found that supernatants from fungi grown in complex media for 2 weeks, filtered and stored for 6 months at 4 ^{o}C continued to give consistent results. It also was useful in identifying mice that were exposed to whole killed cells, rather than spores. Clearly, Applicant's preparation is different from Taylor et al..

As noted on page 549, Material and Methods, left column, 2/3 of the way down, in the processing of their culture supernatants, Taylor et al. dialyzed against water (this would lose components of less than 8,000 mw, aflatoxins are about 400 mw) and freeze-dried them. As discussed, Applicant found that this destroys the antigens according to the instant invention.

Accordingly, it is concluded that Taylor et al. were looking for different things (e.g. sprouting conidia) with different antigens (dialyzed and freeze-dried) than Applicant's claimed invention. The cited reference is not believed to anticipate the presently claimed invention and withdrawal of the Section 102 rejection of the noted claims over Taylor is requested.

In view of the foregoing, it is respectfully submitted that the claims are in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

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